Paper No. 32

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte KOJI HAYASHI, TOSHIYUKI SAKAKI, YOSHIYASU YABUSAKI, KOICHIRO KOMAI, HIDEO KANEKO, and IWAO NAKATSUKA

Appeal No. 2001-0288
Application No. 08/277,031

ON BRIEF

Before WINTERS, SCHEINER, and ADAMS, Administrative Patent Judges.

ADAMS, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on the appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 1-14, which are all the claims pending in the application.

Claims 1, 6, 8, and 10 are illustrative of the subject matter on appeal and are reproduced below:

1. A method for evaluation of the safety of a chemical compound, which comprises:

   (a) reacting the chemical compound with recombinant yeast cells that produce human cytochrome P450 molecular species P450 1A2, P450 2C9, P450 2E1 and P450 3A4 and

a yeast NADPH-P450 reductase, wherein said yeast NADPH-P450 reductase is optionally in the form of a fused enzyme with each of said human cytochrome P450 molecular species, or reacting the chemical compound with cell free extracts of the yeast cells; and

(b) analyzing the resulting metabolite to determine the safety of the compound.

6. A method according to claim 1, wherein the recombinant yeast cells further produce at least one additional human cytochrome P450 molecular species selected from a group of human cytochrome P450 2A6, P450 2C19 and P450 2D6.

8. An artificial fused enzyme, which comprises human cytochrome P450 3A4 and yeast NADPH-P450 reductase.

10. A method of determining in vitro the potential human metabolite of a chemical compound, which comprises:

(a) reacting the chemical compound with recombinant yeast cells that produce human cytochrome P450 molecular species P450 1A2, P450 2C9, P450 2E1 and P450 3A4 and a yeast NADPH-P450 reductase, wherein said yeast NADPH-P450 reductase is optionally in the form of a fused enzyme with each of said human cytochrome P450 molecular species, or reacting the chemical compound with cell free extracts of the yeast cells; and

(b) identifying the resulting metabolite.

The references relied upon by the examiner are:

Yabusaki et al. (Yabusaki)	5,114,852[1]	May 19, 1992
Crespi et al. (Crespi)	WO 92/07085	Apr. 30, 1992
Wolf et al. (Wolf)	WO 92/14817	Sep. 3, 1992

Yasumori et al. (Yasumori '87), "Nucleotide sequence of a human liver cytochrome P-450 related to the rat male specific form," J. Biochemistry, Vol. 102, pp. 1075-1082 (1987)

[1] We note the Final Rejection (Paper No. 27, mailed September 14, 1998), and Brief (Paper No. 29, received December, 9, 1998), rely on Yabusaki et al., United States Patent No. 5,436,159 ('159) and not United States Patent No. 5,114,852 ('852). It appears that the examiner's reference to '852 in the Answer is a typographical error. Furthermore, we note that '852 is a continuation of '159 and therefore both patents have identical disclosures. Therefore, to the extent the examiner's reliance on '852 is in error, we find the error harmless.

Yasumori et al. (Yasumori '89), "Expression of a human P-450IIC gene in yeast cells using galactose-inducible expression system," Molecular Pharmacology, Vol. 35, pp. 443-449 (1989)

Eugster et al. (Eugster), "Constitutive and inducible expression of human cytochrome P450IA1 in yeast Saccharomyces cerevisiae: an alternative enzyme source for in vitro studies," Biochemical and Biophysical Research Communications, Vol. 172, No. 2, pp. 737-744 (1990)

Renaud et al. (Renaud), "Expression of human liver cytochrome P450 IIIA4 in yeast," European J. Biochemistry, Vol. 194, pp. 889-896 (1990)

Sakaki et al. (Sakaki), "Expression of bovine cytochrome P450c21 and its fused enzymes with yeast NADPH-cytochrome P450 reductase in Saccharomyces cerevisiae," DNA and Cell Biology, Vol. 9, pp. 603-614 (1990)

Bligh et al. (Bligh), "Production of cytochrome P450 reductase yeast-rat hybrid proteins in Saccharomyces cerevisiae," Gene, Vol. 110, pp. 33-39 (1992)

Ellis et al. (Ellis), "Catalytic activities of human debrisoquine 4-hydroxylase cytochrome P450 (CYP2D6) expressed in yeast," Biochemical Pharmacology, Vol. 44, No. 4, pp. 617-620 (1992)

Paolini et al. (Paolini), "Wide spectrum detection of precarcinogens in short-term bioassays by simultaneous superinduction of multiple forms of cytochrome P450 isoenzymes," Carcinogenesis, Vol. 12, No. 5, pp. 759-766 (1991)

GROUNDS OF REJECTION

Claims 1-5, 8, 9, 11, 13 and 14 stand rejected under 35 U.S.C. § 103 as being unpatentable over Crespi, Sakaki, Yasumori '89 and Paolini in view of Wolf, Yasumori '87 and Yabusaki.

Claims 6 and 7 stand rejected under 35 U.S.C. § 103 as being unpatentable over Crespi, Sakaki, Yasumori '89 and Paolini in view of Wolf, Yasumori '87 and Yabusaki and further in view of Ellis, Bligh and Eugster.

Claims 10 and 12 stand rejected under 35 U.S.C. § 103 as being unpatentable over Crespi, Sakaki, Yasumori '89 and Paolini in view of Wolf, Yasumori '87 and Yabusaki and further in view of Renaud.

We affirm.

CLAIM GROUPING

Appellants set forth five claim groupings: I, claims 1-5 and 10-14; II, claim 6; III, claim 7; IV, claim 8; and V, claim 9. With regard to group I, since claims 1-5 and 10-14 stand or fall together, we limit our discussion to representative independent claim 1. Claims 2-5 and 10-14 will stand or fall together with claim 1. In re Young, 927 F.2d 588, 590, 18 USPQ2d 1089, 1091 (Fed. Cir. 1991).

DISCUSSION

THE REJECTION OF CLAIMS 1-5, 8, 9, 11, 13 AND 14; AND CLAIMS 10 AND 12

Claim 1:

According to the examiner (Answer, page 4) Crespi teaches "the preparation of a human cell line that concurrently … expresses a set of human cytochrome P450 species – IA2, IIEI and IIIA4 – that are 'primarily responsible for the activation of the major procarcinogens'." The examiner also finds (id.) that Crespi teaches the use of the cells to perform "'assays designed to detect genotoxic effects of promutagens and procarcinogens.'" The examiner further finds (id.) that in addition to IA2, IIEI and IIIA4 Crespi teaches "that 'it is reasonable to expect that additional P450s may be established to have primary responsibility for the activation of other procarcinogens' and that further cDNAs encoding other cytochromes P450 may be expressed in their transformed cells." The examiner, however, recognizes (id.) that Crespi does "not use yeast cells as hosts for the recombinant expression of a set of human cytochromes P450 known to contribute to the metabolic conversion of procarcinogens." In addition,

while Crespi expressly identifies IA2, IIEI and IIIA4, and suggests including other P450 cytochromes, Crespi does not expressly teach cytochrome 2C9.

To make up for this deficiency the examiner relies on Wolf, Sakaki, Yasumori '87, and Yasumori '89. According to the examiner (Answer, page 4) Wolf teaches:

> that Saccharomyces transformants may be alternatively and advantageously used for the recombinant expression of any mammalian cytochrome P450 in such assays so long as a suitable cytochrome P450 reductase is also expressed, preferably as "a hybrid, fusion protein comprising" both the cytochrome P450 and the reductase, which may be a mammalian reductase or a host cell reductase: a "yeast reductase."

To emphasize the teaching of cytochrome P450:NADPH-cytochrome P450 reductase fusion polypeptides, the examiner finds (Answer, page 5) that Sakaki teaches "the recombinant expression in Saccharomyces transformants of two different fusion polypeptides comprising either of two mammalian cytochrome P450s, each fused to a yeast NADPH-cytochrome P450 reductase…."

The examiner finds (Answer, page 6) that Yasumori '89 teaches a human cytochrome P450 "which they designate a 'human-2' cytochrome … [that is] encoded by a cDNA having greatest similarity to the cDNA encoding human cytochrome P450 IIC9, see footnote 1 at page 443…." To further support this teaching in Yasumori '89, the examiner finds (Answer, page 13) that footnote 1 of Yasumori '89:

> indicates that Yasumori et al.[]('89) had already aligned the DNA sequence of Yasumori et al.[]('87), which is SEQ ID NO:2 of the specification, with cDNAs encoding cytochromes P450 known at that time and determined that it was a cytochrom[e] P450 IIC9 cDNA, making it clear that the standard nomenclature for the "human-2" enzyme of [Yasumori '89] would be IIC9.

According to the examiner (Answer, page 12) Yasumori '89 teaches "that expression of the human cytochrome P450 IIC9 plays … 'a substantial role in xenobiotic and carcinogen metabolisms in human liver', where its expression is constitutive, i.e., continuous, and that it can, and should, be recombinantly expressed in yeast."

In view of these teachings, the examiner finds (Answer, pages 6 and 7):

> It would have been obvious to one of ordinary skill in the art at the time the invention was made to supplant the multiple cytochrome P450-expressing transformed human cell of Crespi et al. with the less expensive, more easily-maintained, and specifically regulated yeast transformants which recombinantly express the cytochrome P450/yeast NADPH-cytochrome P450 reductase fusion of Sakaki et al., replacing a bovine cytochrome P450-encoding region of Sakaki et al. in the fusion gene construct with each of the human cytochrome P450 IA2-, IIE1- and IIIA4-encoding DNAs used by Crespi et al. as well as the human cytochrome P450 IIC9-encoding cDNA of Yasumori et al. ('89). This is because Crespi et al. and Yasumori et al. ('89) teach that the human IA2, IIE1, IIA4 and IIC9 cytochromes P450 are all important components in the metabolism of carcinogenic compounds and because Yasumori et al. ('87) teach that the IIC9 species is constitutively expressed – thus is continuously present – in human liver cells. Use of a yeast expression system is further obvious because Sakaki et al. had already provided such a system, with appropriate expression vectors, for recombinant expression of active cytochromes P450 in yeast transformants. One of ordinary skill in the art at the time the invention was made would have experienced motivation to transform yeast cells with expression vectors for the recombinant production of mammalian cytochromes P450 because Wolf et al. had suggested that a system using yeast transformants would be useful.

In response appellants argue (Brief, page 9) that Yasumori '89 "refers to a 'human-2' protein and cDNA … at the time the present invention was made,

there were at least 19 2C subtypes of human cytochrome P450. Thus, where is the motivation to select P450 2C9?" However, as explained by the examiner (Answer, page 13) "[t]he footnote at the bottom of page 443 indicates that Yasumori et al.[](‘89) had already aligned the DNA sequences … making it clear that the standard nomenclature for the ‘human-2’ enzyme of [Yasumori ‘89] would be IIC9." Thus, the motivation to select P450 2C9 comes from Yasumori ‘87 and Yasumori ‘89. A prior art reference must be considered in its entirety in an obviousness inquiry and must include a "full appreciation of what such reference fairly suggests to one of ordinary skill in the art." In re Wesslau, 353 F.2d 238, 241, 147 USPQ 391, 393 (CCPA 1965). We note that appellants did not respond to this point of fact.

Nevertheless, appellants argue (Brief, page 9) even if Yasumori [‘89] teaches the 2C9 cytochrome, "Crespi discloses yeast expressing cytochrome P450s 1A1, 1A2, 2A3, 3A4 and 2E1. Thus, to arrive at the invention … one would have to modify the Crespi disclosure not only to add the 2C9 enzyme but also to suppress expression of the 1A1 and 2A3 enzymes." This argument, however, requires that the claim be read as if the transitional phrase "consisting of" modifies the cytochrome P450 molecular species recited in the claim. It does not, therefore, we cannot agree with appellants’ interpretation of the claimed invention. As explained by the examiner (Answer, page 13) claim 1 "permit[s] the practice of a method that ‘comprises’ the use of the four human cytochromes P450 recited therein, thus [claim 1] neither excludes the use of other

cytochromes P450 in the method nor requires any subtractive modification of the teachings of the prior art." We agree.

Appellants next argue that the specification contains evidence of unexpected results. Specifically, appellants argue (Brief, page 10) "the specification indicates that cytochrome P450 2C9 shows high activity in hydroxylating tolbutamide, P450 2C19 shows good activity, and other P450 2C subtypes show only modest activity." According to appellants (id.):

> though there is a reference indicating that tolbutamide is metabolized by P450 2C subtypes, their relative activity is not expected in view of the cited prior art … [therefore] [t]he selection of P450 2C9 unexpectedly provides a yeast that can best metabolize tolbutamide in addition to other compounds and this unexpected result provides a ground for patentability of the invention described in claims 1-5 and 10-14.

We are not persuaded by appellants' argument. It is well settled that once a prima facie case of obviousness is established, the burden of going forward with proofs of patentability shifts to the applicant. In re Rinehart, 531 F.2d 1048, 1051, 189 USPQ 143, 147 (CCPA 1976). However, it is equally well settled that the comparison must be with the closest prior art, In re De Blauwe, 736 F.2d 699, 705, 222 USPQ 191, 195 (Fed. Cir. 1984); In re Burckel, 592 F.2d 1175, 1179, 201 USPQ 67, 70 (CCPA 1979); and that the comparison must be commensurate with the scope of the claims, In re Grasselli, 713 F.2d 731, 743, 218 USPQ 769, 778 (Fed. Cir. 1983). On this record, claim 1 does not require that the yeast metabolize tolbutamide. Instead, as the examiner explains (Answer, page 14) the limitation in claim 1 drawn to the "'evaluation of the safety of a chemical compound', requires no particular efficacy with any single

compound." In addition, the examiner points out (id.) that the results relied upon in the specification are from yeast transformed with a single mammalian cytochrome P450, which is substantially different from the yeast transformants claimed, and taught by the combination of prior art relied on, that "require the simultaneous expression of at least four mammalian cytochromes P450." Accordingly, on this record, appellants failed to meet their burden.

On reflection, we find no error in the examiner's rejection of claim 1. As discussed supra claims 2-5 and 10-14 fall together with claim 1. We note that appellants grouped claims 10 and 12 together with claims 2-5, 11, 13 and 14, which as explained above fall together with claim 1. Under these circumstances we find it unnecessary to enter into a discussion of Crespi, Sakaki, Yasumori '89 and Paolini in view of Wolf, Yasumori '87 and Yabusaki and further in view of Renaud.

Accordingly we affirm the rejection of claims 1-5, 11, 13 and 14 under 35 U.S.C. § 103 as being unpatentable over Crespi, Sakaki, Yasumori '89 and Paolini in view of Wolf, Yasumori '87 and Yabusaki; and the rejection of claims 10 and 12 under 35 U.S.C. § 103 as being unpatentable over Crespi, Sakaki, Yasumori '89 and Paolini in view of Wolf, Yasumori '87 and Yabusaki and further in view of Renaud.

Claim 8:

According to appellants (Brief, page 14) "[t]he invention of claim 8 is a fusion protein between human cytochrome P450 3A4 and a yeast cytochrome P450 reductase." In addition, appellants recognize (id.) that Crespi "discloses

expression of human P450 3A4 in human cells … [and] [t]he Wolf reference discloses generically that mammalian P450 enzymes can be fused to yeast reductase and the fusion can be expressed in yeast cells." Nevertheless, appellants argue that since none of the references "specifically exemplifies" the claimed fusion, there is "no motivation provided in the cited prior art to produce" the fusion. We note that appellants fail to identify any authority upon which to support this assertion.

In our opinion, appellants' argument lacks merit, "[t]he test for obviousness is not express suggestion of the claimed invention in any or all of the references but rather what the references taken collectively would suggest to those of ordinary skill in the art presumed to be familiar with them." In re Rosselet, 347 F.2d, 847, 851,146 USPQ 183,186 (CCPA 1965). Stated differently, a specific exemplification is not necessary. Therefore, on this record, we find no error in the examiner's rejection. Accordingly, we affirm the rejection of claim 8 under 35 U.S.C. § 103 as being unpatentable over Crespi, Sakaki, Yasumori '89 and Paolini in view of Wolf, Yasumori '87 and Yabusaki

Claim 9:

According to appellants (Brief, page 15) "[t]he invention recited in claim 9 [is drawn to] a yeast expression plasmid comprising DNA encoding a fusion protein." Appellants present two arguments in response to the examiner's rejection. First, relying on the same rationale asserted for claim 8, supra, appellants argue (Brief, page 15), "the cited references provide no motivation to

make the specific combination recited in claim 9." For the reasons set forth for claim 8, supra, we are not persuaded by appellants' argument.

Second, appellants argue (Brief, bridging paragraph, pages 15-16) with reference to In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991) and Ex parte Ochiai, 24 USPQ2d 1265 (Bd. Pat. App. & Int. 1992) that "the expression of proteins in heterologous systems, as exemplified by the present instance of expression of a human protein in a yeast cell, is one considered by the USPTO to be inherently unpredictable." Therefore, "[a]ppellants submit [Brief, page 16] that the inherent unpredictability of heterologous gene expression makes the invention set forth in claim 9 unobvious over the cited references."

We cannot agree with appellants' argument. As explained by the examiner (Answer, page 18) "this proposition … ignore[s] the clear difference between the pertinent facts in Vaeck and the facts at issue in the instant application." Furthermore to the extent that appellants would argue that the cited case law stands for the proposition that a per se rule exits, we point out that, since the decisions in Bell and Deuel, our appellate reviewing court has made it clear that there are no per se rules of obviousness or nonobviousness. In re Ochiai, 71 F.3d 1565, 1572, 37 USPQ2d 1127, 1133 (Fed. Cir. 1995) ("reliance on per se rules of obviousness is legally incorrect.") Accord, In re Brouwer, 77 F.3d 422, 425, 37 USPQ2d 1663, 1666 (Fed. Cir. 1996).

On reflection, we find no error in the examiner's rejection. Accordingly, we affirm the rejection of claim 9 under 35 U.S.C. § 103 as being unpatentable over

Crespi, Sakaki, Yasumori '89 and Paolini in view of Wolf, Yasumori '87 and Yabusaki

CLAIMS 6 AND 7

We note that the examiner relies on the teachings of Crespi, Sakaki, Yasumori '89, Paolini, Wolf, Yasumori '87 and Yabusaki as applied to claims 1-5, 8, 9, 11, 13 and 14, supra. However, the examiner now emphasizes (Answer, page 8) that Crespi teaches that "cytochrome P450 1A1 should be recombinantly expressed among multiply expressed cytochromes P450 in a transformed cell line to detect carcinogenic metabolites of compounds…." The examiner also finds (id.) that Eugster "teach that the human cytochrome P450 1A1 may be successfully expressed in yeast transformants in active form and is involved in the metabolism of 'a large group of promutagens present as ubiquitous environmental pollutants' but is present at high levels in liver cells only when induced…." According to the examiner (id.) Ellis "teach that the human cytochrome P450 IID6 may be successfully expressed in yeast transformants in active form and that its metabolism has been studied in association with 'diseases such as cancer and Parkinson's disease.'" In addition, the examiner finds (id.) that Bligh "teach that the human cytochrome P450 IIA6 may be successfully expressed in yeast transformants in active form and may be studied … in the metabolism of 'carcinogen activation and deactivation'." According to the examiner (Answer, page 9) it would have been obvious to combine Eugster, Ellis, and Bligh teachings of human P450 cytochromes that are implicated in the metabolism of carcinogen formation and/or drug metabolism with the

combination of Crespi, Sakaki, Yasumori '89, Paolini, Wolf, Yasumori '87 and Yabusaki. We note that Crespi teaches "that 'it is reasonable to expect that additional P450s may be established to have primary responsibility for the activation of other procarcinogens' and that further cDNAs encoding other cytochromes P450 may be expressed in their transformed cells."

Claim 6:

According to appellants (Brief, page 12) "the Eugster, Ellis and Bligh references merely provide description of the 2A6 or 2D6 enzymes. None of the references provide any description at all of the 2C19 enzyme." Appellants also argue (id.) that "with respect to the 2C19 enzyme, the specification provides evidence of unexpected results obtained using this enzyme." However, as the examiner points out, the claim "recites a Markush group of alternate, or even multiple, choices. Patentability of this claim may not be determined solely by the presence or absence of expression of a cytochrome P450 IIC19 when the limitations of the claim are satisfied by any one of a set of equivalent elements." We agree.

Appellants also argue (Brief, page 12) that there is no suggestion to combine the 2A6, 2C19 or 2D6 enzymes with the 1A2, 2C9, 2E1 and 3A4 enzymes "to make a yeast that is useful in a method for assaying the safety of a chemical compound." In response, the examiner argues (Brief, page 15) that Ellis:

> teach that the human cytochrome P450 IID6 may be successfully expressed in yeast transformants in active form and that its metabolism has been studied in association with "diseases such as cancer and Parkinson's disease." Bligh et al. teach that the human

> cytochrome P450 IIA6 may be successfully expressed in yeast transformants in active form and may be studied … in the metabolism of "carcinogen activation and deactivation."

According to the examiner (id.) "[b]oth teachings thus supply ample motivation to utilize the recombinant expression of either cytochrome P450 in a method to evaluate the safety of a chemical compound…." We note that the test of obviousness is "whether the teachings of the prior art, taken as a whole, would have made obvious the claimed invention." In re Gorman, 933 F.2d 982, 986, 18 USPQ2d 1885, 1888 (Fed. Cir. 1991). On this record, we find no error in the examiner's rejection.

Accordingly, we affirm the rejection of claim 6 under 35 U.S.C. § 103 as being unpatentable over Crespi, Sakaki, Yasumori '89 and Paolini in view of Wolf, Yasumori '87 and Yabusaki and further in view of Ellis, Bligh and Eugster.

Claim 7:

According to appellants (Brief, page 13) "the Eugster … references [sic] merely provide description of the 1A1 enzyme. None of these references describe the 2B6, 2C8 or 2C18 enzymes. Therefore appellants conclude (id.) "with respect to these three enzymes, even if the references are combined as suggested by the [e]xaminer, the result is not the claimed invention." However, as the examiner explains (Answer, page 16) claim 7 recites a Markush group of enzymes, which includes 1A1 as taught by the combination of references relied upon.

Appellants further argue (Brief, bridging paragraph, pages 13-14) that "none of these references provide any suggestion that one or more of the 1A1,

2B6, 2C8 or 2C18 enzymes should be combined with the 1A2, 2C9, 2E1 and 3A4 enzymes to make a yeast that is useful in a method for assaying the safety of a chemical compound." We are not persuaded by appellants' argument. As discussed supra Crespi disclose yeast expressing cytochrome P450s 1A1, 1A2, 2A3, 2E1 and 3A4. When Crespi is combined with the teachings of Sakaki, Yasumori '89 and Paolini in view of Wolf, Yasumori '87 and Yabusaki as in the rejection of claim 1, a person of ordinary skill in the art would have had a method as set forth in claim 1 wherein the recombinant yeast cells produce 1A1, 1A2, 2A3, 2C9, 2E1 and 3A4. Therefore, having found no error in the examiner's rejection of claim 1, we find no error in the examiner's rejection of claim 7. Accordingly, we affirm the rejection of claim 7 under 35 U.S.C. § 103 as being unpatentable over Crespi, Sakaki, Yasumori '89 and Paolini in view of Wolf, Yasumori '87 and Yabusaki and further in view of Ellis, Bligh and Eugster.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 CFR § 1.136(a).

AFFIRMED

SHERMAN D. WINTERS)
Administrative Patent Judge)
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)
) BOARD OF PATENT
TONI R. SCHEINER)
Administrative Patent Judge) APPEALS AND
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) INTERFERENCES
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